ACTIVATION OF PHAGOCYTOSIS IN VITRO WITH FRACTIONS OF FASEOLOSAXIN

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The investigations of many authors show that the phagocytic activity of leucocytes can be stimulated by natural and synthetic substances (1, 2, 5, 6, 8, 9). The possible action of phytohaemagglutinin (PHA) upon the phagocytosis is the object of numerous recent studies. Tzoneva and Boshnakova (5, 6) in 1967 found that the application of 30 mcg/ml PHA Faseolosaxin increases considerably the phagocytic activity of human peripherial leucocytes in vitro. The authors presume this quality as a result of the immunological properties of Faseolosaxin.

It is a Bulgarian PHA, isolated from the seeds of beansort Saks by using the method of M. Tzoneva (7). Faseolosaxin (PHA-F) is produced regularly in seria and is widely applied in the cytogenetic practice in our country. The substance is heterogenic and 3 protein fractions are isolated until now: F_1, F_2, F_3 (3). Their role is still unrevealed concerning the changed phagocytic activity under the influence of PHA.

The object of our work is to study the effect of PHA-F and its fractions upon the leucocytic phagocytosis in vitro (rabbit blood).

Material and methods

Experimental solutions with a concentration of 20 mcg/ml were prepared from liophylizated substance of the investigated preparations. This dose was comformable with the percentage content of the fractions of the total preparation. 1 ml of the corresponding solution of the preparations was added to 3 ml heparinized rabbit blood and the samples were incubated at 37° C for 30 min. After that 1 ml alive bacterial suspension in saline solution (Staph. aureus with density of 2 mld/ml) was added. After a 15-minute additional incubation smears were prepared and stained after Gimsa (5, 6). The phagocytic monocytes and segmentonuclear cells were counted on each 100 intact leucocytes which presents the ratio "percent phagocytosis". The average number of cocci, phagocytated by 1 phagocyte, was presented by the "phagocytic index". 600 cells were counted of each experimental series. The statistical reliance of the received results compared to the controls was calculated by using the methods of variational and alternative analysis.

Results and discussion

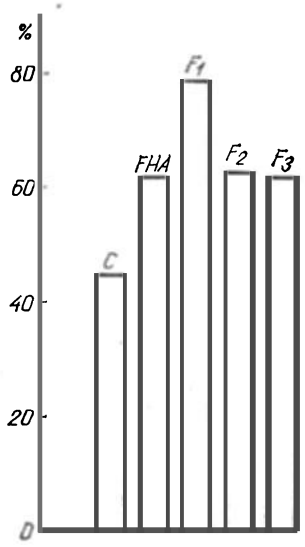
Table 1 shows the experimental data. It is obvious that the applied substances considerably stimulate the phagocytic activity of the leucocytes in vitro.

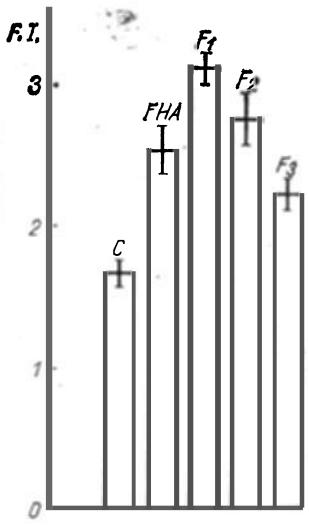
Table 1

Higher percent of phagocytosis with PHA-F and its fractions

Preparation	% Phagocytosis	p	Phagoc. index	p
Controls	45.92±3.69	—	1.66±0.10	—
PHA-F	62.42±3.59	0.01	2.55±0.09	0.001
F_1	78.92±3.02	0.01	3.12±0.13	0.001
F_2	63.92±3.56	0.01	2.76±0.21	0.001
F_3	63.25±3.57	0.01	2.23±0.12	0.001

The analysis of the results shows that fraction "F_1", is most active with highest elevation of the phagocytic index and percent phagocytosis. Concerning both indexes, the fractions are graduated as follows: $F_1 > F_2 > F_3$.





Graph 1: Increased phagocytotic index under the influence of the applied preparations

Graph 2: Nuclear phagocytosis with leucocytes treated by PHA

The same relation of the fractional effect is established also in other investigations of their biological activity (4). It is quite interesting to confront it to the data of forming of nutritive vacuoles in Paramecium caudatum under the influence of PHA-F and its fractions (4). This kind of phagocytosis of the unicellular organisms is similar to our data, concerning the comparative results of the effect of fractions.

The graphs N_0 1 and N_0 2 show that there is a considerable difference between the action of the control and the preparations themselves, while the difference between the individual fractions is not statistically reliable.

Treating of leucocytes with fractions of PHA-F tends to a new cell reactivity. We establish the phenomenon "nuclear phagocytosis" quite

often — cocci included (swallowed) in the phagocytes' nuclei. Tzoneva and Boshnakova (2, 3) found similar phenomena, including amitosis of lymphocytic phagocytosis, under the influence of the total preparation PHA-F upon human blood. We also found lymphocytic amitosis with F_1 — a probable budding. It is interesting, that in lymphocytic culture, F_1 rises the percent of the cells, divided by amitotic way (4). Our data also confirm the peculiar effect of F_1 upon blood cells.

The present results show that by treating of leucocytes in vitro with fractions of PHA-F, the phagocytosis is remarkably activated. F_1 has the strongest effect and it has the quality of a well-purified phytohaemagglutinin. The investigations of our study add certain details to the common phenomenon of the biological action of the preparation Faseolosaxin and its fractions.

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АКТИВИРОВАНИЕ ФАГОЦИТОЗА ИН ВИТРО ФРАКЦИЕЙ ФАЗОЕОЛОСАКСИНА

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РЕЗЮМЕ

Исследовано влияние трех изолированых фракции болгарского фитогемаглютанина фазеолоксина $Ф_1$, $Ф_2$, $Ф_3$ на фагоцитоз ин витро. Определен процент фагоцитоза и фагоцитарного индекса 600 фагоцитов из каждой опытной постановки. При концентрации 20 мкг/мл отдельные фракции вызывают значительные изменения в активности фагоцитов крови кроликов. Наиболее сильным стимулирующим действием обладает фракция $Ф_1$. При действии фазеолоксаксином и его фракциями на лейкоциты проявляется и качественно новая реактивность кровяных клеток: ядреный фагоцитоз и амитоз лимфоцитов. Эти исследования дополнительно выясняют общую картину биологического действия препарата фазеолосаксина.